CrCDPK1, a predicted lipid-binding domain in the N terminus of the protein, is required for CrCDPK1 enrichment at the flagellar tip and proximal half of the flagellum, suggesting that concentration of CrCDPK1 at these regions requires an association with the flagellar membrane. Furthermore, CrCDPK1 redistributes during flagellar assembly; this indicates that CrCDPK1 localization is dynamic and that CrCDPK1 itself could potentially be transported to the flagellar tip, in an inactive form, by kinesin-II-driven anterograde IFT. In this scenario, kinesin-II would carry its own "deactivator" to the flagellar tip, where CrCDPK1 would then be activated, phosphorylate FLA8, and promote kinesin-II dissociation from the IFT particle.

Finally, if kinesin-II dissociates from the IFT particle at the flagellar tip, how is kinesin-II recycled back to the flagellar base? It is possible that at least some of the kinesin-II motor could diffuse back to the flagellar base. Consistent with this, direct visualization of kinesin-II by total internal reflection fluorescence microscopy of *Chlamydomonas* cells expressing KAP-GFP revealed multiple anterograde IFT tracks but very few retrograde IFT tracks (Engel et al., 2009). The study by Liang et al. (2014) sets the stage for further investigation into the intriguing and largely unexplored mechanisms that control IFT and ciliary assembly.

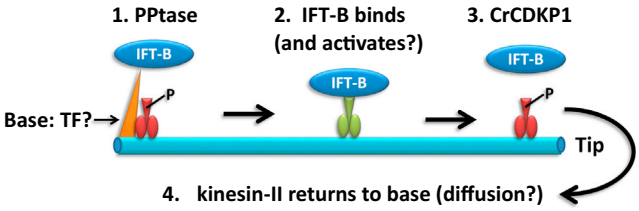

Figure 1. Phosphoregulation of IFT Kinesin-II
Model depicting the findings of Liang et al. and some open questions regarding regulation of kinesin-II by FLA8 phosphorylation. At the ciliary base, possibly at the transition fibers (TFs), an unknown phosphatase (PPtase) dephosphorylates FLA8 (step 1). This allows IFT complex B (IFT-B) to bind to kinesin-II (step 2), which then translocates to the ciliary tip. It remains to be determined whether kinesin-II motor activity is stimulated by dephosphorylation, IFT-B binding, another mechanism, or a combination of events. When kinesin-II reaches the tip, CrCDKP1 phosphorylates FLA8 (step 3), causing IFT-B to dissociate from kinesin-II. If kinesin-II doesn't return to the base via IFT, it might diffuse back (step 4). CrCDKP1 may have additional functions at the tip, e.g., activation of dynein-mediated retrograde transport to return IFT particles to the base of the cilium.

REFERENCES

Berman, S.A., Wilson, N.F., Haas, N.A., and Lefebvre, P.A. (2003). Curr. Biol. *13*, 1145–1149.

Collingridge, P., Brownlee, C., and Wheeler, G.L. (2013). Curr. Biol. *23*, 2311–2318.

Deane, J.A., Cole, D.G., Seeley, E.S., Diener, D.R., and Rosenbaum, J.L. (2001). Curr. Biol. *11*, 1586–1590.

Engel, B.D., Lechtreck, K.F., Sakai, T., Ikebe, M., Witman, G.B., and Marshall, W.F. (2009). Methods Cell Biol. *93*, 157–177.

Guillaud, L., Wong, R., and Hirokawa, N. (2008). Nat. Cell Biol. *10*, 19–29.

Hilton, L.K., Gunawardane, K., Kim, J.W., Schwarz, M.C., and Quarmby, L.M. (2013). Curr. Biol. *23*, 2208–2214.

Liang, Y., Pang, Y., Wu, Q., Hu, Z., Han, X., Xu, Y., Deng, H., and Pan, J. (2014). Dev. Cell *30*, this issue, 585–597.

Rosenbaum, J.L., and Witman, G.B. (2002). Nat. Rev. Mol. Cell Biol. *3*, 813–825.

Tam, L.W., Ranum, P.T., and Lefebvre, P.A. (2013). Mol. Biol. Cell *24*, 588–600.

Wren, K.N., Craft, J.M., Tritschler, D., Schauer, A., Patel, D.K., Smith, E.F., Porter, M.E., Kner, P., and Lechtreck, K.F. (2013). Curr. Biol. *23*, 2463–2471.

Lipids Guide the Way: Targeting Proteins to the Chloroplast Outer Envelope Membrane

Lynn G.L. Richardson,[1,2] Yamuna D. Paila,[1,2] and Danny J. Schnell[1,*]
[1]Department of Biochemistry and Molecular Biology, Life Sciences Laboratories Room N431, 240 Thatcher Road, University of Massachusetts, Amherst, MA 01003-9364, USA
[2]Co-first authors
*Correspondence: dschnell@biochem.umass.edu
http://dx.doi.org/10.1016/j.devcel.2014.08.017

Correct delivery of peptides to appropriate subcellular organelles requires distinct trafficking and targeting mechanisms. In this issue of *Developmental Cell*, Kim et al. (2014) demonstrate that AKRA2, a targeting receptor for chloroplast outer envelope membrane proteins, binds chloroplast-specific lipids to ensure proper delivery of cargo to the chloroplast outer envelope.

Organelle biogenesis and function in all eukaryotic cells rely on highly specific targeting pathways to direct thousands of proteins from the cytosol to the proper subcellular compartment. Over the past two decades, the machinery and targeting signals responsible for the import of proteins across boundary membranes into the ER, mitochondria, peroxisomes, and chloroplasts have been extensively studied (Wickner and Schekman, 2005). In each case, intrinsic

targeting signals within nascent or newly synthesized proteins are recognized by specific receptors in the cytoplasm or at the organelle surface. The targeting receptors are coupled to multimeric membrane complexes that mediate membrane translocation into the organelle. However, a unique set of membrane proteins is inserted directly into the boundary membranes of organelles from the cytoplasm, and their targeting involves components distinct from the canonical protein import systems (Denic, 2012). The transmembrane domains (TMDs) of these membrane proteins contain key elements for targeting specific organelles, raising the question of how the targeting determinants and the components of the targeting pathways have evolved to ensure delivery of proteins to the appropriate boundary membrane. In this issue of *Developmental Cell*, Kim et al. (2014) provide new insight into how chloroplast outer membrane (COM) proteins are targeted to the organelle and thereby avoid insertion into the boundary membranes of the endoplasmic reticulum (ER), mitochondria, or peroxisomes.

In this study, the authors demonstrate that ankyrin repeat protein 2A (AKR2A), a cytosolic receptor for COM proteins, specifically targets COM proteins to chloroplasts by recognizing the unique lipid composition of the outer envelope membrane. The authors previously demonstrated a central role for AKR2A/B in chloroplast biogenesis in *Arabidopsis thaliana*, as knockdown or knockout of AKR2A and its close homolog AKR2B, respectively, resulted in defects in chloroplast development and outer envelope membrane targeting (Bae et al., 2008). AKRA2 recognizes a specific TMD flanked by positively charged residues in order to recruit intended protein substrates (Bae et al., 2008). The positively charged region distinguishes the TMD targeting signal of COM proteins from other substrates and prevents mistargeting to the ER (Lee et al., 2011). While the C-terminal ankyrin repeat domain (ARD) of AKR2A was known to mediate chloroplast localization (Bae et al., 2008), an interaction that was enhanced by the small heat shock protein sHsp17.8 (Kim et al., 2011), it was unclear how this domain associates with chloroplasts and whether a chloroplast outer envelope receptor or receptors may exist to recognize AKR2A at the chloroplast surface.

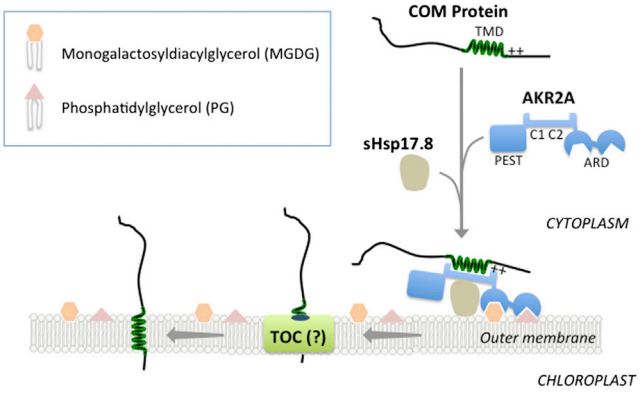

Figure 1. A Model for AKR2A-Mediated Targeting of Membrane Proteins to the Outer Envelope Membrane of Chloroplasts

The transmembrane domain (TMD) and adjacent charged region of newly synthesized chloroplast outer membrane proteins (COM) are recognized by AKR2A in the cytoplasm via the central domains (C1 and C2) of the receptor. Delivery of the AKR2A-COM protein complex to the chloroplast surface is mediated by binding of the AKR2A C-terminal ankyrin repeat domain (ARD) to the chloroplast-specific lipids, monogalactosyldiacylglycerol (MGDG) and phosphatidylglycerol (PG), thereby ensuring selective targeting to the chloroplast. The small heat shock protein 17.8 (sHsp17.8) assists in targeting. Insertion of the COM protein into the outer membrane is proposed to be facilitated by a component of the TOC complex, which also mediates protein import into the organelle (Lee et al., 2013).

In this paper, the authors demonstrate that AKR2A specifically recognizes monogalactosyldiacylglycerol (MGDG) and phosphatidylglycerol (PG) at the outer membrane for docking and delivery of membrane protein cargo (Figure 1). Duramycin-induced aggregation of MGDG within envelope membranes reduced AKR2A binding to the COM. Similar results were observed in mutants with reduced levels of MGDG or PG synthesis, providing compelling evidence for a coordinate role of these lipids in AKR2A targeting. When outer envelope vesicles were derived from these mutants, exogenous supplementation with appropriate lipids led to increased binding of AKR2A in a dose-dependent manner, demonstrating that lipid composition of the vesicles mediates AKRA2 binding. Furthermore, the authors demonstrated that AKR2A binding was insensitive to trypsin treatment of chloroplasts, suggesting that AKR2A binding to chloroplasts does not require proteinaceous components at the envelope surface in addition to MGDG and PG.

AKR2A has four conserved regions, including the ARD domain known to mediate chloroplast targeting (Bae et al., 2008). To gain mechanistic insight into this activity, the authors determined the X-ray crystal structure of the ARD domain. The structure revealed three adjacent grooves with clusters of hydrophobic and polar residues similar to other known lipid binding proteins (Chen et al., 2012). Mutation of selected residues within two of these grooves, L_1 and L_2, reduced MGDG- or PG-dependent binding of AKR2A, respectively.

Phylogenetic analysis showed that the ARD is highly conserved in land plants and certain green algae but is distinct from ARD domains of cyanobacteria, suggesting that AKR2A evolved from a minimal ARD-containing protein of the green algae-derived host cell. The authors proposed that additional conserved N-terminal domains were added stepwise to AKRA2 during evolution, resulting in specificity of AKR2A for its chloroplast membrane protein substrates.

Organelle-specific lipids contribute to the unique identity of cellular compartments, facilitating the sorting of proteins during membrane trafficking and functioning as receptors for the recruitment of specific enzymes and signaling molecules (Holthuis and Menon, 2014). The finding that AKR2A mediates chloroplast outer envelope targeting by specifically recognizing MGDG—a unique lipid of the chloroplast outer envelope (Block et al., 1983)—is an exciting development in understanding the mechanisms by which lipids function in the selective targeting of membrane proteins to specific organelles. It is of great interest to understand how targeting proteins to the chloroplast surface is coupled to downstream events mediating their insertion into the membrane bilayer. Previous studies have shown that the protein import channel of the translocon of the outer membrane

of chloroplasts (TOC) facilitates insertion of COMs into the outer membrane, suggesting that the AKR2A targeting pathway and the TOC protein import pathway might converge to complete the targeting cycle (Lee et al., 2013). By contrast, the insertion of ER tail-anchored (TA) proteins, another class of membrane proteins directly inserted into boundary membranes, appears to be facilitated by unique components of the guided entry to TA proteins (GET) pathway that function downstream of the cytosolic targeting components (Denic, 2012). Specific components for outer membrane protein targeting in mitochondria remain to be identified, and the nature of the pathway that ensures the fidelity of targeting and insertion of membrane proteins to this organelle is also of great interest.

REFERENCES

Bae, W., Lee, Y.J., Kim, D.H., Lee, J., Kim, S., Sohn, E.J., and Hwang, I. (2008). Nat. Cell Biol. *10*, 220–227.

Block, M.A., Dorne, A.J., Joyard, J., and Douce, R. (1983). J. Biol. Chem. *258*, 13281–13286.

Chen, Y., Sheng, R., Källberg, M., Silkov, A., Tun, M.P., Bhardwaj, N., Kurilova, S., Hall, R.A., Honig, B., Lu, H., and Cho, W. (2012). Mol. Cell *46*, 226–237.

Denic, V. (2012). Trends Biochem. Sci. *37*, 411–417.

Holthuis, J.C.M., and Menon, A.K. (2014). Nature *510*, 48–57.

Kim, D.H., Xu, Z.Y., Na, Y.J., Yoo, Y.J., Lee, J., Sohn, E.J., and Hwang, I. (2011). Plant Physiol. *157*, 132–146.

Kim, D.H., Park, M.-J., Gwon, G.H., Silkov, A., Xu, Z.-Y., Yang, E.C., Song, S., Song, K., Kim, Y., Yoon, H.S., et al. (2014). Dev. Cell *30*, this issue, 598–609.

Lee, J., Lee, H., Kim, J., Lee, S., Kim, D.H., Kim, S., and Hwang, I. (2011). Plant Cell *23*, 1588–1607.

Lee, D.W., Jung, C., and Hwang, I. (2013). Biochim. Biophys. Acta *1833*, 245–252.

Wickner, W., and Schekman, R. (2005). Science *310*, 1452–1456.